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### MORPHOLOGY AND ULTRASTRUCTURE OF THE PYGIDIAL GLAND OF THE ANT *DINOPONERA AUSTRALIS* (HYMENOPTERA, FORMICIDAE)

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#### ABSTRACT

*The pygidial glands of Dinoponera australis appear as two very large cell clusters underneath the anterior margin on either side of the seventh tergite. The polygonal secretory cells are associated with slender duct cells, that carry the secretion into the reservoir, which is formed by the invaginated intersegmental membrane between the 6th and 7th tergites. Ultrastructural examination reveals the occurrence of a vesicular smooth endoplasmic reticulum, a well developed Golgi apparatus and abundant mitochondria. These characteristics may correspond with the elaboration of a pheromonal secretion. The pygidial gland in this species is probably involved in its generalized recruitment system.*

**Keywords:** *Dinoponera australis*, morphology, pygidial gland, ultrastructure.

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## INTRODUCTION

Social insects in general and ants in particular are characterized by an overwhelming variety of exocrine glands that are found all over their bodies, and that amount to a total of 39 different glands for the Formicidae only (Billen, 1994). Among the abdominal glands, one group is associated with the sting apparatus, another is formed by glands that are associated with the tergites and sternites. Additional, but much smaller intersegmental glands, can be classified as tergo-sternal, dorsolateral and lateroventral in position (Jessen & Maschwitz, 1983). The pygidial gland is the most conspicuous tergal gland in ants, and occurs as a paired structure that opens through the intersegmental membrane between the 6th and 7th tergites. It is extremely developed among the Dolichoderinae (Pavan & Ronchetti, 1955).

The pygidial glands in general are involved in the secretion of pheromonal substances, although there is no common characteristic function for the Formicidae in general. Among the several known pheromonal functions are the elaboration of alarm substances (Pavan & Ronchetti, 1955; Kugler, 1979), trail pheromones (Simon & Hefetz, 1991), recruitment pheromones (Maschwitz & Schönegge, 1977), sex pheromones (Hölldobler & Haskins, 1977), and smearing substances (Jessen & Maschwitz, 1983). A survey of the general appearance of the pygidial gland in ants is given by Hölldobler & Engel (1978), illustrating its widespread occurrence in members of all subfamilies, including the Formicinae, where it was previously thought not to occur (Hölldobler, 1984). Ultrastructural data dealing with the pygidial gland so far are restricted to a description of the gland in Dolichoderinae (Billen, 1986).

Queenlessness is a widespread phenomenon in ponerine ants, and has recently received intense attention, especially as to the reproductive differentiation and social organization (see revision in Peeters, 1991). The role of exocrine glands in the biology of queenless species, however, is poorly known. Oldham *et al.* (1994) compared the volume and contents of the mandibular glands of all members of a colony of *Dinoponera australis* with the reproductive status of the workers, noting a large difference in the amount of secretion between the gamergate (mated worker) and its non-mated nestmates.

In the present paper, we report on the morphology and ultrastructure of this gland in the queenless ponerine ant *D. australis*.

## MATERIAL AND METHODS

A nest of *Dinoponera australis* was excavated at Itirapina, SP, Brasil, and the pygidial glands of all individuals found (the gamergate and 12 workers)

were fixed in 2% cold glutaraldehyde, buffered at pH 7.3 in 50 mM Na-cacodylate and 150 mM saccharose and postfixed in 2% cold osmium tetroxide in the same buffer. After dehydration in a graded acetone series, the glands were embedded in araldite and sectioned with a Reichert Ultracut E microtome. Sections were stained in a LKB 2168 Ultrastainer and examined in a Zeiss EM900 electron microscope. Semithin sections were stained with methylene blue and thionin. Glands for scanning microscopy were fixed in the same way as tissues for transmission microscopy, after which they were dehydrated in a graded ethanol series and critical point dried in a Balzers CPD 030 instrument, and viewed in a Philips SEM515 microscope.

### RESULTS

The pygidial gland in *Dinoponera australis* comprises a left and a right very large elongate cluster of polygonal secretory cells (diameter approx. 40–50  $\mu\text{m}$ ) with rounded nuclei, underneath the anterior margin of the 7th abdominal tergite, bilaterally disposed (Fig. 1). Each cluster measures approx. 800  $\mu\text{m}$  on an abdominal cross section, by 150  $\mu\text{m}$  on a longitudinal section (Fig. 2). A rough count leads to an estimate of approximately 300 secretory cells per cluster. The glandular cells are individually connected through asso-

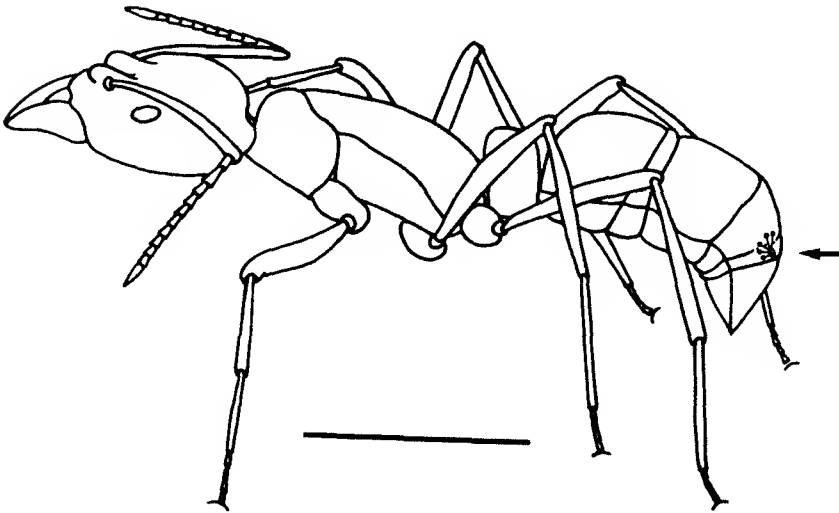


Fig. 1. Schematic drawing of a *Dinoponera australis* worker showing the position of the left pygidial gland cluster (arrow). Scale bar 0,5 cm.

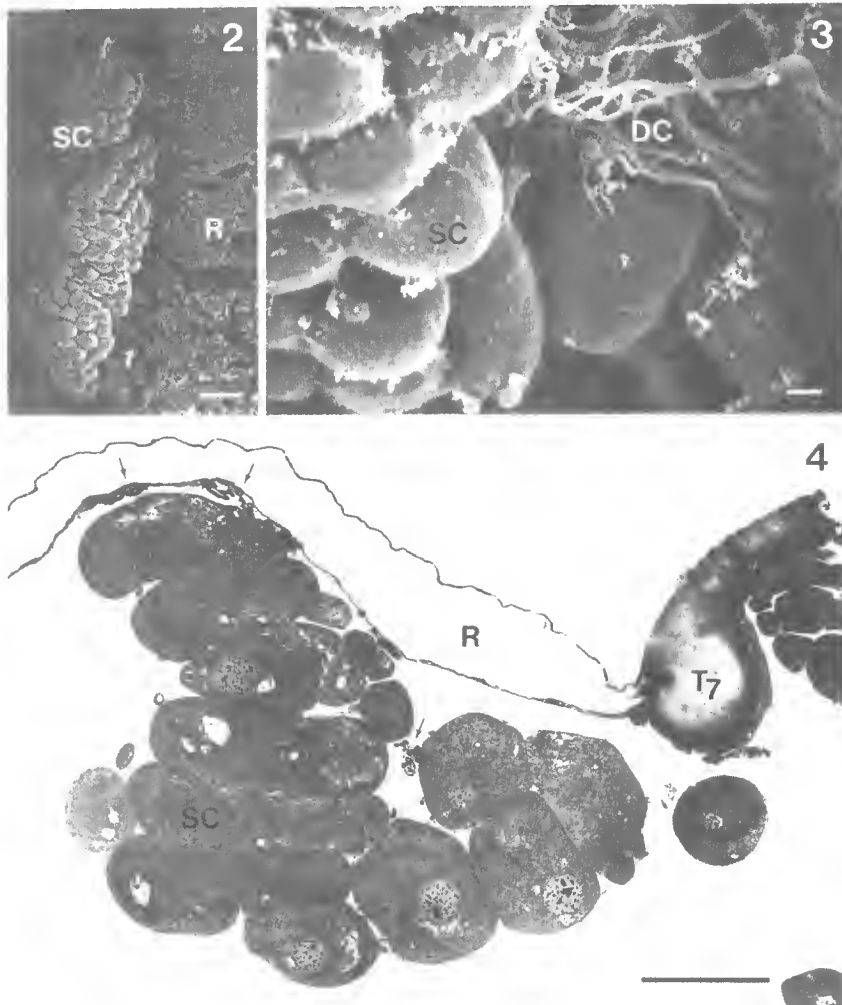


Fig. 2. Scanning electron micrograph of a pygidial gland, showing the secretory cell clusters (SC) with ducts (arrow) opening into the reservoir (R). Scale bar 100  $\mu$ m; 3. Detail of secretory cells (SC) and duct cells (DC) (scanning electron micrograph, scale bar 10  $\mu$ m); 4. Longitudinal semi-thin histological section through pygidial gland secretory cells (SC) and duct cells (arrows). R: reservoir wall (intersegmental membrane between 6th and 7th tergites), T7: anterior margin of seventh abdominal tergite (scale bar 50  $\mu$ m).

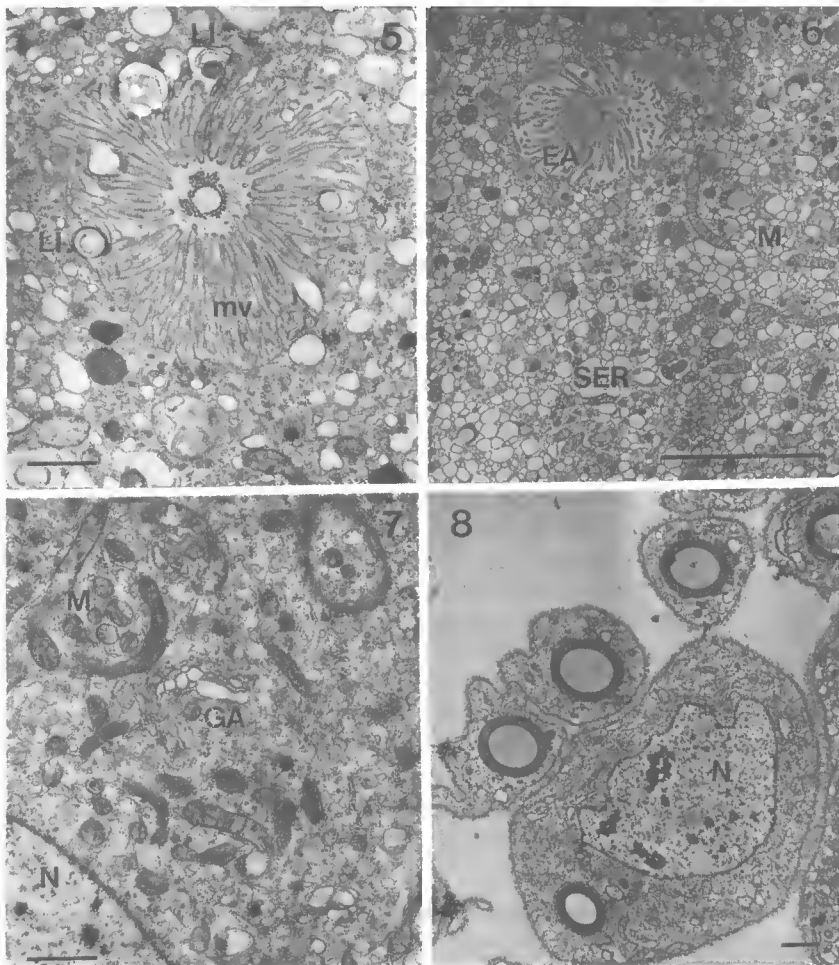
ciated duct cells with the pygidial gland reservoir (Figs. 3, 4), which is formed by an invagination of the intersegmental membrane between the 6th and 7th abdominal tergites. As this invagination is rather narrow, however, the reservoir has a relatively small volume. There is no muscular supply associated with the gland nor with its reservoir.

Each secretory cell is characterized by the intracellular end apparatus, which consists of a microvillar sheath surrounding the central cuticular duct, which has an internal diameter of approx.  $0.4\ \mu\text{m}$ . The cuticular lining of the duct is characterized by a discontinuous electron-dense epicuticle (Fig. 5). The microvillar pattern may display an orderly arrangement (Fig. 5) or may show conspicuous extracellular spaces that force the microvilli to occur in a more loose pattern (Fig. 6). The cytoplasm is characterized by an abundance of mitochondria and a well developed Golgi apparatus (Fig. 7), as well as an elaborate smooth endoplasmic reticulum of the vesicular type (Fig. 6). Spherical lamellar inclusions with a diameter around  $1\ \mu\text{m}$  can be observed, especially in the region of the end apparatus, where they occur in between the microvilli (Fig. 5). The duct cells are characterized by a much reduced cytoplasm, and a continuous cuticular duct with an internal diameter around  $1\ \mu\text{m}$  and a cuticular lining of approx.  $0.2\ \mu\text{m}$  (Fig. 8).

We were not able to find any significant difference between the gland and reservoir of the gamergate and the non-mated workers. In the laboratory and field conditions, we observed several times pairs of workers in tandem, that is, a worker following a leader worker, keeping physical contact with it by means of slight and repeated touches of the antennae to the gaster dorsum of the leader.

#### DISCUSSION

The pygidial gland represents one of the most conspicuous exocrine elements in the giant Neotropical ant *Dinoponera australis*. The gland is formed by numerous bicellular units, each consisting of a secretory cell and its accompanying duct cell, which therefore correspond to the class 3 glandular cells following the classification of Noirot & Quennedey (1974). These are characterized by the presence of an end apparatus, that represents the junction between the secretory cell and the duct cell. Size variation of the extracellular space that occurs at this junction, due to differences in the arrangement of the microvilli of the end apparatus, can probably be seen as an expression of a different stage in the cell's secretory activity, as has also been observed in other exocrine glands (Bazire-Bénazet & Zylberberg, 1979). The possible formation, for each secretory cell individually, of an additional reservoir space,



Figs. 5-8 (scale bar  $1\mu\text{m}$ ); 5. Detail of the end apparatus, showing the fenestrated epicuticular layer of the central duct, and lamellar inclusions (LI) in between the microvilli (mv); 6. Cytoplasm with end apparatus (EA), mitochondria (M) and wide cisterns of smooth endoplasmic reticulum (SER); 7. Cytoplasmic detail showing Golgi apparatus (GA) and mitochondria (M). N: nucleus; 8. Cross section through duct cells. N: nucleus.

allows temporary storage of secretory products, which may be important for glands with a rather small common reservoir, as in the present case of the pygidial gland of *D. australis*. In Dolichoderinae, on the other hand, the pygidial gland reservoir is extremely enlarged. For this reason, this gland has

been erroneously interpreted as an 'anal gland' (Pavan & Ronchetti, 1955; Billen, 1986). In the Myrmicinae, the gland appears to be more developed in the more advanced genera (Kugler, 1978).

The cytoplasm of the secretory cells contains a very well developed smooth endoplasmic reticulum and Golgi apparatus, and an abundance of mitochondria. These characteristics are typical of pheromone producing glands, where non-proteinaceous molecules of low molecular weight are elaborated (Noirot & Quennedey, 1974; Billen, 1991). The lamellar inclusions probably represent the secretory products, as is also illustrated by their occurrence near to or even in between the microvilli of the end apparatus, where they may be on their way to the reservoir (Hefetz & Orion, 1982; Billen, 1986).

The observation of frequent tandem running, with contacts between individuals involving the place where the pygidial glands are located, the absence of a muscular supply associated with the gland or its reservoir, and the absence of volatile substances in the gland and its reservoir, suggest that the pygidial gland in *Dinoponera australis* may be constantly producing and releasing contact pheromones of relatively low volatility, employed in their generalized recruitment system. Also the lack of significant differences between the pygidial gland of gamergates and non-mated workers suggests that this gland is not involved in reproduction.

According to Hölldobler and Wilson (1990), the secretions of the pygidial glands in ponerine ants can function as tandem running pheromones, recruitment and trail pheromones, or sex pheromones, depending on the species and the behavioural context. In *Pachyncondyla obscuricornis*, Traniello and Hölldobler (1984) suggested that the secretion of the pygidial gland appears to be transferred to the hindlegs of a tandem pair leader by a series of self-grooming behaviours that occur prior to the formation of a tandem pair. In this species the formation of tandem pairs is related to nest migration, which can also be the case of *Dinoponera*, as observed in *D. gigantea* by Overal (1980). In spite of a presumable involvement in the production of pheromonal substances based on the ultrastructural characteristics, however, preliminary chemical analysis of the pygidial glands of *D. australis* could not confirm the presence of any volatile compounds (Oldham & Morgan, pers. comm.).

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